Verification of the Occurrence of the α_{s1}-Casein A Allele in Red Danish Cattle [1]

Abstract

α_{s1}-Casein A, a rare genetic polymorph of the α_{s1}-casein fraction of cow's milk, probably arose through the sequential deletion of eight amino acid residues. The α_{s1}-A allele was thought to occur only in American Holstein cattle; however, it has been reported to occur in low frequency in Red Danish cattle. No ancestral relationship can be found between Red Danish cattle and the Holstein breed, hence further confirmation of the identity of these two polymorphs was sought. The proteins were isolated by DEAE-cellulose chromatography and the fingerprints of their chynotryptic digests compared. The α_{s1}-A variant from the Red Danish breed proved to be identical with that of the Holstein breed where α_{s1}-A was first observed. Therefore, it is proposed that this mutant form of α_{s1}-casein is of more ancient ancestry, since no relationship between the Red Danish and the American Holstein breeds can be documented.

Introduction

Thompson and his colleagues (11) reported the discovery of genetic polymorphism in α_{s1}-casein, the major protein component of cow's milk. Subsequently it was established that α_{s1}-A, the rarest of the variants of the α_{s1}-casein series, differs from the predominant B variant by the sequential deletion of no less than eight amino acids from the protein molecule (9). The gene for α_{s1}-A was thought to be a mutation of recent origin, limited to a single bloodline of American Holstein cattle. Since these original observations, two additional reports of the occurrence of the A variant have emerged. One report (3) concerned the discovery of two animals heterozygotic for the variant (A/B) in New Zealand Holsteins. The α_{s1}-A from New Zealand Holsteins was confirmed by one of us (MPT) to exhibit electrophoretic mobility indistinguishable from that of α_{s1}-A from American Holsteins. A logical explanation for the occurrence of the α_{s1}-A gene in New Zealand can be made by import of American Holstein cattle. However, the second report does not lend itself to such a simple explanation; it concerns the appearance (5) of α_{s1}-A in three Red Danish cows heterozygous for the variant. It is difficult to see any possible relationship (1) between American Holstein cattle and the Red Danish breed originating from North Slesvig Red (+ Angeln and Ballum) × local island (6). In addition, the A variant has not been found in any other European breed of cattle or in Indian and African zebu cattle (2). We regarded the discovery of the A variant in the Red Danish breed as tenuous, since the sole basis of identity with Holstein α_{s1}-A was relative mobility upon gel electrophoresis. To test the identity of these two α_{s1}-A variants, fingerprints of their chymotryptic digests were compared, because it had already been established that authentic α_{s1}-casein A exhibits a characteristic chymotryptic peptide fingerprint which distinguishes it from the B and C (4) or D variants (unpublished results).

Experimental Procedures

DEAE-Cellulose column chromatography of the caseins was performed as previously described (8).

The purity of the isolated α_{s1}-A from Red Danish cattle was verified by standard methods of gel electrophoresis (8). The α_{s1}-casein A samples from Red Danish and U.S. Holstein cattle were digested with chymotrypsin and subsequently fingerprinted under the controlled conditions described for the caseins by Kalan et al. (4).

Results and Discussion

Although no Red Danish cows homozygous for the A variant were available, we were able, by chromatography on DEAE-cellulose in the presence of urea (8), to fractionate successfully the α_{s1}-A variant from a casein phenotyped α_{s1}-AD (Fig. 1). Theoretically, the chromatographic separation of α_{s1}-A from B or C should be simpler than the separation of α_{s1}-A from D (on gel electrophoresis, α_{s1}-B and C are more clearly resolved from A than is the D variant). Such, however, is not the case. We have never been able to resolve the AB mixture.

The chymotryptic fingerprints of the isolated α_{s1}-caseins A from U.S. Holstein and Red Danish cattle are compared in Figure 2. The absence of the peptide labelled 8 (characteristically missing in α_{s1}-A) and the appearance of the peptide labelled "27" (present in the A, but not in the B, C or D variants), coupled

[1] Rødt Dansk Malkevaeg.

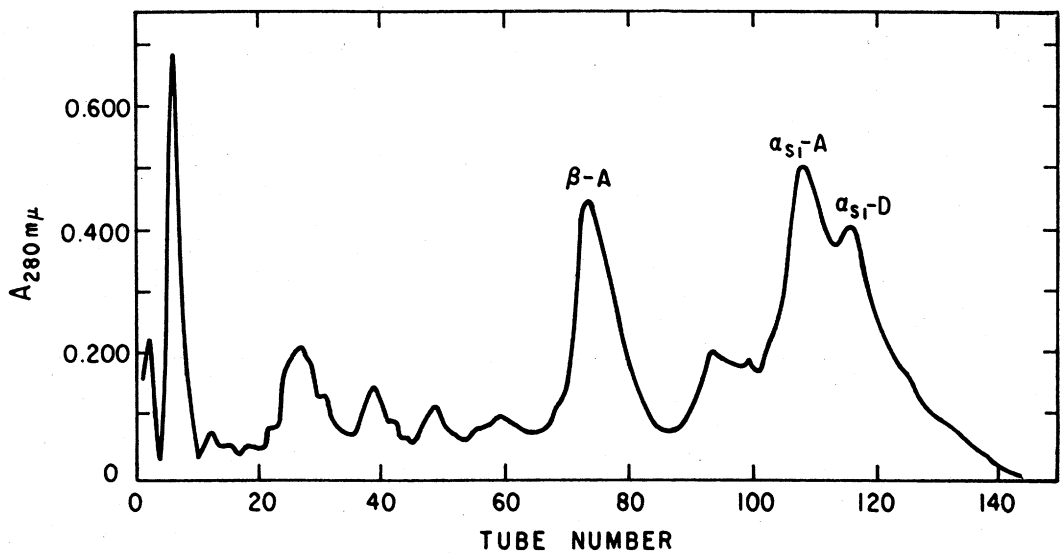

FIG. 1. Separation of α_{s1}-A from Red Danish cattle by DEAE-cellulose column chromatography. Whole casein sample was phenotyped α_{s1}-AD and chromatography performed in the presence of urea and mercaptoethanol, with a gradient of 0 to 0.3 M NaCl as previously described (8).

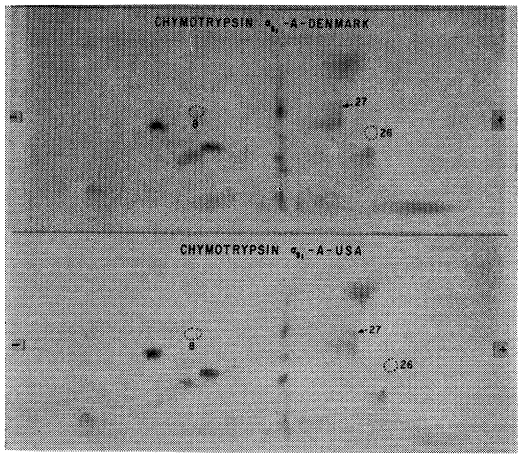

FIG. 2. Comparison of the chymotryptic fingerprints of α_{s1}-caseins A from Red Danish and U.S. Holstein cattle. Two-dimensional peptide maps were prepared as previously described by Kalan et al. (4). Two A variants appear to be identical; note the absence of Peptides 8 and 26, which normally appear in α_{s1}-B, C and D, and the occurrence of Peptide 27 peculiar to α_{s1}-A. Numbers correspond to those previously assigned (4).

with the absence of the peptide labelled 26, provides strong evidence that α_{s1}-caseins A from the milks of American Holstein and Red Danish cattle are identical.

As previously stated, no relationship between American Holstein and Red Danish cattle is known. That this mutant form of α_{s1}-casein could have arisen independently in two different breeds of cattle is somewhat remarkable, since the deletion of eight amino acid residues in the α_{s1}-A molecule involves the deletion of 24 bases in the DNA. We had considered the α_{s1}-A gene to be of recent origin; however, these results allow us to speculate that the A gene is of more ancient ancestry.

It is improbable that the nursing calf would suffer any particular nutritional disadvantage from the α_{s1}-casein A; however, it is evident that the physical-chemical properties of the α_{s1}-A protein (7, 10) render it an undesirable component for conventional milk processing. Hence, one could suggest that the gene was inadvertently "selected against" in animal breeding; it occurs in very low frequency, < 0.03, in both Holstein and Red Danish cattle. Alternatively, the observation of a major deletion of eight amino acid residues in a protein molecule, occurring in milks of two distinct breeds of cattle, suggests some common ancestry, although no documentary evidence is available.

In conclusion, α_{s1}-casein A offers one of the best examples known of a large-scale sequential deletion of amino acids within a polypeptide chain, and its occurrence in two such unrelated breeds of cattle is difficult to explain.

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